

THE LIFE-HISTORY OF *TRYPANOSOMA GAMBIENSE* AND *TRYPANOSOMA RHODESIENSE* AS SEEN IN RATS AND GUINEA-PIGS*

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PLATE XXVII.

CONTENTS		PAGE
Introduction	...	465
Methods	...	466
Résumé of previous work on Latent Bodies of Trypanosomes	...	467
The Relation between Latent Bodies and the number of Trypanosomes in the Peripheral Blood, with Notes on the Parasites found in the Internal Organs of the Hosts	...	468
The Formation of Latent Bodies from Flagellate Trypanosomes	...	472
The Structure of Latent Bodies	...	474
The Metamorphosis of Latent Bodies into Trypanosomes	...	476
The Significance of the Non-flagellate or Latent Forms of Trypanosomes. Inoculation with Latent Bodies produces Trypanosomiasis	...	477
Note on the Degeneration of Trypanosomes	...	480
Summary and Conclusions	...	481
References	...	482
Explanation of Plate	...	484

INTRODUCTION

The researches recorded in this paper were undertaken at the suggestion of Major Ross, who wished me to investigate the parasitological aspect of the numerical cyclical development discovered by him and Dr. D. Thomson (1910) in the trypanosome occurring in a patient suffering from sleeping sickness contracted in Rhodesia, particularly as regards the possible connection of the latent bodies of Salvin-Moore and Breinl (1907) with that cycle. The investigations have been conducted in the Liverpool School of Tropical Medicine, under a grant from the Tropical Diseases Research Fund.

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A complete and generally accepted life cycle of *Trypanosoma gambiense* has yet to be written. The following paper is offered as a contribution to the solution of this difficult problem, and deals with that portion of the life history of the parasite which takes place in a vertebrate host.

The sub-inoculations in animals—rats and guinea-pigs—recorded herein were made from a patient suffering from Rhodesian sleeping sickness in Professor Ross's clinic in the Royal Southern Hospital, Liverpool. The trypanosome from this source showed a marked morphological feature in the possession of a posterior nucleus in some forms, and for this parasite the name *T. rhodesiense* has been suggested by Stephens and Fantham (1910). A laboratory strain of *T. gambiense* was also used in these investigations for comparison.

Special attention has been paid to the observation of the *living* parasite, as well as to stained preparations.

METHODS

Fresh preparations of *T. gambiense* and *T. rhodesiense* were made from blood taken aseptically from animals, namely, tame rats and guinea-pigs. The blood was kept in sealed cover-slip preparations, at or below blood heat (25° to 37° C.), and also at laboratory temperature. The blood was sometimes diluted with a little physiological salt solution or with a little isotonic sodium citrate solution. Methylene blue was sometimes used for *intra vitam* staining.

Wet preparations of the parasite were made on cover-slips after fixation with osmic acid vapour or corrosive acetic alcohol. The chief stains used were iron haematoxylin and those of Giemsa and Romanowsky.

Dry smears were employed for rough work.

In animals killed at certain stages of infection, the various internal organs were carefully examined both in fresh preparations and stained smears.

The enumerative methods used were those recently employed by R. Ross and D. Thomson and used by various workers in Liverpool, being an elaboration of R. Ross's thick-film method made with measured quantities of blood (1 cubic millimetre divided

into quarters). The films were dehaemoglobinised, fixed in absolute alcohol, and stained by the Romanowsky method.

RÉSUMÉ OF PREVIOUS WORK ON LATENT BODIES OF TRYPANOSOMES

The various flagellate forms of *Trypanosoma gambiense* have been so often described by many competent workers that it is needless to discuss them further in detail. Suffice it to say that long, thin trypanosomes may occur, especially at the beginning of infection in rats and guinea-pigs, and shorter, stout or stumpy forms later. Trypanosomes intermediate in character also are found.

The so-called rounded, latent or encysted forms, which are non-flagellate, must be discussed in greater detail. Marked attention to these non-flagellate forms of trypanosome was first drawn by Moore and Breinl (1907-8) in mammalian trypanosomes, though Dutton saw rounded forms of an amphibian trypanosome on the Congo in 1903-5. Moore and Breinl (1907), working on stained material, stated that latent forms occurred in the internal organs, especially during the periods when the flagellates were decreasing or absent from the peripheral blood of the host. These authors give a curve showing the variations in the numbers of *T. gambiense* in infected rats; they do not, however, give any numerical data in support of the graph. A few other workers have mentioned rounded bodies in connection with trypanosomes, among whom Hindle (1909) may be noted. The significance of the latent bodies is still a disputed point, and it was recently stated in a review that more evidence was required to show that they 'constitute part of a life-cycle in the vertebrate host.'*

In the present paper the general statements of Moore and Breinl, regarding forms of *T. gambiense* in the internal organs of rats, are shown to be accurate, and many details, as seen in the living parasite, as well as numerical data from daily counts of the trypanosomes, are supplied in proof of the significance of the latent bodies. Animals inoculated with *T. rhodesiense* (Stephens and Fantham) have also been carefully investigated.

* S.S. Bureau, Bulletin No. 15 (March, 1910), p. 102.

**THE RELATION BETWEEN LATENT BODIES AND THE NUMBER
OF TRYPANOSOMES IN THE PERIPHERAL BLOOD, WITH
NOTES ON THE PARASITES FOUND IN THE
INTERNAL ORGANS OF THE HOSTS**

Following on the work of R. Ross and D. Thomson (1910) on the enumeration of the parasites in the peripheral blood of a patient suffering from Rhodesian sleeping sickness, the periodic increase in the number of trypanosomes in the peripheral blood of rats and guinea-pigs inoculated with *T. rhodesiense* or with *T. gambiense* has recently been found by Fantham and J. G. Thomson.

Rats similarly inoculated and exhibiting such periodic increase and decrease were killed at various points in the cycle, as set forth in the following tables:—

A. RAT 1.—*T. gambiense*, Laboratory Strain, in Piebald ♂ Rat, weight 150 grams, inoculated with 50,000 trypanosomes.

Day	1	2	3	4	5	6	7	8
Parasites per c.mm.	—	—	—	—	—	—	4	1088
Temp.*	55	46	70	...	62	57	54

Day	9	10	11	12	13	14	15
Parasites per c.mm.	4392	868	1656	2560	9288	6304	436 killed
Temp.*	60	68	44	46	57	54	61

* The temperature is expressed according to the following convention, as in Major Ross's recent papers on 'Malaria' and 'Trypanosomiasis':—Temp. = $(F - 95) \times 10$, where F is the temperature in degrees Fahrenheit, recorded with a clinical thermometer.

Heart blood of Rat 1 examined fresh, immediately after killing.—A few living trypanosomes seen and some (fewer) rounded forms.

Spleen.—Very large; three to seven rounded forms seen in every field of the microscope (2 mm. objective and 2 ocular); no flagellates seen in fresh preparation.

Liver and portal blood.—Living flagellates and rounded bodies seen in about equal numbers (one of each per field).

Lungs.—A few living flagellates seen, four times as many rounding and rounded bodies seen [100 parasites counted in this and subsequent fresh preparations, when possible].

Bone marrow.—Many rounded bodies.

RAT 2.—*T. gambiense*, Laboratory Strain, in White ♀ Rat, weight 128 grams, inoculated with 200,000 trypanosomes.

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	—	4	684	3640
Temp.	38		40	45	50	54	60

Day	8	9	10	11	12
Parasites per c.mm.	7392	10,000	6320	1840	2200, killed
Temp.	55	58	44	46	48

The internal organs of Rat 2 were in much the same condition as in Rat 1; further details are superfluous.

B. RAT 3.—*T. rhodesiense* in Piebald Rat, ♀, weight 108 grams, inoculated with 350,000 trypanosomes.

Day	1	2	3	4	5	6
Parasites per c.mm.	—	—	172	18,160	42,120	31,040
Temp.	22	20	20	40	56	36

Day	7	8	9	10
Parasites per c.mm.	21,600	12,844	130,000	61,440, killed
Temp.	20	21	15	14

Heart blood, fresh, of Rat 3.—Free trypanosomes seen.

Spleen.—Many rounded bodies; no free trypanosomes seen.

Liver.—A few free trypanosomes seen.

Lungs.—About equal numbers of flagellates and rounded bodies.

Bone marrow.—Rounded bodies.

RAT 4.—*T. rhodesiense* in White Rat, ♂, weight 120 grams, inoculated with 600,000 trypanosomes.

Day	1	2	3	4	5	6
Parasites per c.mm.	—	—	4	896	2880	31,360
Temp.	20	25	38	30	30

Day	7	8	9	10	11
Parasites per c.mm.	51,200	64,000	22,820	12,500	6144, killed
Temp.	48	47	13	20	31

Heart and liver of Rat 4.—Many large trypanosomes seen in fresh preparations, very few rounded bodies.

Lungs.—A few free flagellates, three times as many rounded forms.

Spleen.—A few small flagellate trypanosomes (3 per cent.); many rounded forms.

Kidney blood.—Flagellate trypanosomes.

Bone marrow.—Rounded bodies.

RAT 5.—*T. rhodesiense* in White Rat, ♀, weight 247 grams, inoculated with 120,000 trypanosomes.

Day	1	2	3	4	5	6
Parasites per c.mm.	—	—	—	—	4	12
Temp.	52	38	34	96

Day	7	8	9	10
Parasites per c.mm.	20	296	624	3744, killed
Temp.	86	86	20	66

Flagellate trypanosomes were seen in all the internal organs of Rat 5. A few latent bodies were seen in the lungs, spleen, and bone marrow.

RAT 6.—*T. rhodesiense* in White Rat, ♂, weight 150 grams, inoculated with 500,000 trypanosomes.

Day	1	2	3	4	5	6	7	8	9
Parasites per c.mm. ...	—	—	—	—	72	2056	1968	6420	592, killed
Temp.	38	36	51	56	54	70	70	62	54

Heart blood, fresh, of Rat 6.—Flagellate parasites seen, only three latent bodies found in a fresh preparation.

Liver.—Flagellates 96, and latent bodies 4; in one hour, however, the number of latent bodies in the fresh preparation had increased 16 times (namely to 64), and the flagellates were correspondingly fewer.

Lungs.—About equal numbers of trypanosomes and latent bodies.

Spleen.—Many latent bodies, no free flagellates seen.

Bone marrow.—A few latent bodies, no flagellates seen.

RAT 7.—*T. rhodesiense* in Piebald Rat, ♀, weight 170 grams, inoculated with 1,000,000 trypanosomes. Weight 182 grams at death, but pregnant.

Day	1	2	3	4	5	6
Parasites per c.mm. ...		—	8	5852	27,916	16,468, killed
Temp.	38		62	55	67	66

Heart blood of Rat 7.—Many flagellates, few rounded bodies.

Liver.—Equal numbers of flagellates and rounded bodies.

Lungs.—Three times as many latent bodies as flagellates.

Bone marrow.—Large rounded bodies.

Spleen.—No flagellates seen, but many rounded bodies.

Splenic vein blood.—Twice as many flagellates as rounded bodies.

Mesenteric gland fluid.—Many flagellates.

Lymph.—Rounded bodies and Crithidia-like forms.

Placental blood.—Living flagellates, few rounded bodies.

Thirteen embryos.—Serous fluid contained a very few trypanosomes.

Embryonic liver.—No flagellate trypanosomes seen.

From counts of the number of trypanosomes in the peripheral blood daily, and from examination of carefully prepared smears of organs, it is found that latent bodies are most numerous when the flagellate parasites are few. If inoculated animals be killed at these periods, very few flagellate trypanosomes are found in the spleen and bone marrow (see preceding tables), but many latent bodies are present in those organs, while rounding forms are seen especially in the lungs.

In the peripheral blood, on the upward slope of the curve representing the numbers of the parasites from day to day, the parasites increase in numbers by longitudinal division to a maximum. At or about this period the formation of rounded or latent bodies begins, and takes place especially in the internal organs.

If rounded (latent) bodies, derived from the internal organs of an infected rat, be placed in warm fresh blood drawn from a normal, uninfected rat, then growth of some of the rounded bodies towards the flagellate trypaniform stage can be seen under the microscope, as is detailed in a subsequent section of this paper.

Five guinea-pigs (three inoculated with *T. rhodesiense* and two with *T. gambiense*), dying in various stages of trypanosomiasis, were carefully examined, and fresh preparations and smears of their internal organs were made. Rounding and rounded forms of trypanosomes were seen, just as in infected rats.

Needless to say, a very careful examination of the internal organs of normal (uninfected) rats and guinea-pigs was made for the purposes of comparison and control.

THE FORMATION OF LATENT BODIES FROM FLAGELLATE TRYPANOSOMES

This is diagrammatically represented in text-fig. 1, in which the formation of a rounded body from a living trypanosome was observed under the microscope in a drop of blood and lymph from

the tail of an infected rat. The time taken for the formation of the rounded body was thirty minutes.



TEXT-FIG. 1 represents diagrammatically the formation of a latent (non-flagellate) body of *Trypanosoma gambiense* in infected rat's blood as seen under the microscope, on a warm stage, during a period of 30 minutes.

The intervals were 3, 8, 15, 20, 23, 25, and 30 minutes respectively from the commencement of change of form of the parasite. Intranuclear karyosome not represented.

In this process the anterior or flagellar end disintegrates and is cast off, while at the other end the blepharoplast (kinetocore) gradually migrates nearer the nucleus, and then the non-flagellar or posterior end of the original trypanosome is cast off, together with the remains of the flagellum. The rounded body, consisting of chromatin with a thin layer of cytoplasm, has then surrounded itself with a definite, very thin capsule ('cyst'). This process, as seen under the microscope, is either the natural mode of formation of non-flagellate bodies in the internal organs or closely approximates

thereto, as intermediate stages exactly similar in the formation of these bodies are seen in stained preparations of the heart, lungs, and spleen (Plate XXVII, figs. 1-5).

However, in the peripheral blood there occur rounded, oval or somewhat pyriform parasites (figs. 17-20), each with a single anterior flagellum. Such forms may, for convenience, be called rounding herpetomonad forms, as *Herpetomonas* passes through similar stages in assuming a rounded non-flagellate form. The rounded stages of *Herpetomonas*, thus formed, have been aptly termed post-flagellate stages by Captain Patton and by Dr. Annie Porter in their recent interesting researches on flagellates (*Crithidia* and *Herpetomonas*). The rounded, encapsuled stages of trypanosomes are post-flagellate stages. That these rounded, post-flagellate forms are encapsuled in a thin membranous structure is shown by the fact that they resist maceration in water much longer than the trypaniform flagellates.

The formation of post-flagellate bodies (figs. 2, 3, 5) is well seen in the lungs, whence they find their way in the blood stream to the spleen and bone marrow.

THE STRUCTURE OF LATENT BODIES

The post-flagellate (latent) stages of *T. gambiense* and *T. rhodesiense*, already mentioned, have a relatively simple structure. The strictly latent or non-flagellate forms are usually oval in outline, and small, about $2\ \mu$ to $4\ \mu$ in diameter (figs. 2-16). Less frequently are they quite rounded or spherical (fig. 14), while sometimes they are pyriform (figs. 25, 27, 29, 30). Internally there is a nucleus, which may show a karyosome, and beside the nucleus there is a blepharoplast or kinetonucleus (fig. 6). In some latent bodies, especially the smallest ones, the kinetonucleus may not be visible separately (figs. 11, 13-15), as it may be lying over the nucleus, or actually affixed thereto (figs. 2, 10). The juxtaposition of the nuclear bodies has been actually observed in some Romanowsky-stained specimens, after careful wet fixation (figs. 2, 10). The relative positions of the nucleus and blepharoplast in rounding or rounded bodies may vary considerably. A small quantity of cytoplasm occurs in the latent bodies.

In the formation of rounded, latent bodies, as seen *in vitro*, a portion of the body of the flagellate, after passage of the blepharoplast towards the nucleus, is thrown off, and the flagellum is discarded (text-fig. 1). Examination of preparations of the internal organs (lungs, heart, spleen) of the host shows that a similar method of formation of the post-flagellate stages of the parasite usually occurs in the internal organs (figs. 1-5). However, in the peripheral blood, after careful searching of a sufficient quantity of blood ($\frac{1}{4}$ cubic millimetre), a few rounding or rounded parasites can generally be seen (figs. 12, 17-20). Also, in the internal organs, stages of the parasite intermediate between the flagellate and non-flagellate forms may be seen (fig. 33).

The post-flagellate or latent bodies vary in size (figs. 2-16, 22). This variation is due to two causes: (1) the formation of non-flagellate parasites from trypanosomes of different breadths, and (2) the occasional division of large post-flagellate forms by binary fission, an example of the simplest schizogony (fig. 23). This fission, so far as my researches go, is infrequent in the case of *T. gambiense* and *T. rhodesiense*. It has been observed that division of flagellate trypanosomes may immediately precede the formation of latent bodies.

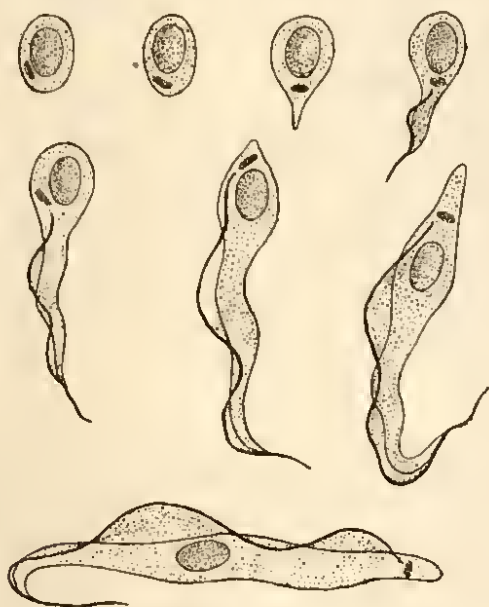
Broad forms of *T. rhodesiense*, with posterior nucleus, may form relatively large latent bodies. As the nucleus is at or near the posterior (non-flagellar) end of the parasite, there is little of the body discarded in that region when rounding occurs. The kidney shape of the nucleus of some specimens of *T. rhodesiense* is seen in their latent bodies (fig. 27), and the nucleus lies to one side of the rounding body (figs. 17, 18). It is not easy, however, to differentiate between the latent bodies of *T. rhodesiense* and *T. gambiense*.

Moore and Breinl describe a stainable band or black line connecting the nucleus and blepharoplast of certain specimens of *T. gambiense* at or near the maxilla. An 'interaction' takes place between the blepharoplast and nucleus. After this the formation of latent bodies proceeds. During the researches now recorded, there was no good evidence found in support of Moore and Breinl's views. The stainable band was seen in some stout (probably old) parasites, and in parasites at periods near the death of the host.

It is possible, as Swellengrebel (1908) suggested, that the stainable line is a form of degeneration. However, on the exact significance of the stainable band seen in such trypanosomes I prefer not to pronounce a definite opinion at present.

THE METAMORPHOSIS OF LATENT BODIES INTO TRYPANOSOMES

This process was observed in life on several occasions, though the complete passage from a rounded body to a fully flagellate moving trypanosome was only rarely seen (three times), for it is difficult to imitate precisely the natural conditions favourable to such a metamorphosis. However, by taking rounded bodies, usually obtained from the spleen of an infected rat, in a little physiological salt solution, and adding thereto an equal quantity of fresh (normal, uninfected) rat's blood, some of the rounded bodies were seen on a warm stage (25° to 35° C.) to grow, each becoming larger and sending out a process (text-fig. 2). This pseudopodium-like process lengthens, and a flagellum is formed from an area close to



TEXT-FIG. 2 represents diagrammatically the metamorphosis of a rounded, latent or non-flagellate parasite into a flagellate trypanosome (*T. gambiense*). The rounded bodies, obtained from the spleen of an infected rat, were placed in warm, fresh, uninfected rat's blood and watched under the microscope. The total time taken for the metamorphosis was about one hour.

the blepharoplast (kinetonucleus). At this stage the parasite forms at its drawn-out anterior end an undulating membrane along the edge of which the flagellum lies, and the organism somewhat resembles a *Crithidia*. This transitory stage may be termed the crithidial stage. The organism grows and the blepharoplast passes posterior to the nucleus, and the trypaniform stage is assumed.

The evidence of stained preparations (figs. 32 to 40) fully supports this mode of formation of flagellate trypanosomes from non-flagellate latent bodies. The latent bodies, which are the post-flagellate stages of one generation of trypanosomes, become the pre-flagellate stages of the succeeding generation of trypanosomes. Such pre-flagellate, *Crithidia*-like parasites in various stages of metamorphosis may be seen in the peripheral blood of the host (figs. 32, 34 to 42), when the parasites are increasing in numbers therein.

A basal granule (blepharoplast of Minchin) is seen at the base of the flagellum of some stained flagellating parasites (fig. 37).

It may be added that in dealing with an isolated case of an intermediate stage of a parasite between the flagellate and the rounded body, it is sometimes difficult to determine whether the given stage is pre-flagellate or post-flagellate, that is, whether the given parasite is proceeding in development towards the flagellate stage or away from it towards the rounded body.

THE SIGNIFICANCE OF THE NON-FLAGELLATE OR LATENT FORMS OF TRYPANOSOMES. INOCULATION WITH LATENT BODIES PRODUCES TRYPANOSOMIASIS

R. Ross and D. Thomson (1910) report periodic variations in the numbers of the trypanosomes found in the blood of a patient, W. A., suffering from Rhodesian Sleeping Sickness. Fantham and J. G. Thomson (1910) report similar periodic variation in the number of the parasites in the peripheral blood of sub-inoculated animals (rats, guinea-pigs, and rabbits). During the periods of decrease of the parasites in the peripheral blood, I find that latent (non-flagellate) bodies are present in relatively large numbers in the internal organs of the host. The latent bodies are formed at or near the period of maximum increase of the trypanosomes in the peripheral blood. The latent bodies are especially numerous in

the spleen and bone marrow on the downward slope of the curve representing the numbers of the parasites in the peripheral blood of the host. Change of the latent forms into trypanosomes takes place on the rise or upward slope of the curve.

There is, of course, a mutual action and reaction of the host and the parasite, the resistance of the host probably being greatest when the flagellate trypanosomes within it are beginning to decrease, thus helping to bring about the assumption of the rounded form by many of the flagellates, so that latent or resistant non-flagellate stages of the parasite are then numerous.

The occurrence of latent bodies also helps to explain the successful inoculation of animals with trypanosomiasis when no flagellates can be found in the blood inoculated from a previously infected animal. Although it might be urged that flagellate trypanosomes in numbers too few to recognise may actually be present in the infected blood inoculated, yet it is possible to inoculate only latent, non-flagellate bodies, and give the inoculated animal trypanosomiasis. In other words, persistent infectivity in the case of trypanosomes is explained by rounded bodies.

I have performed this experiment (inoculation of latent bodies) on two occasions. In the first experiment a rat was inoculated with one drop of spleen-pulp (from Rat 7) mixed with a little sterile physiological salt solution, the mixture containing no flagellates. The inoculated rat developed trypanosomiasis on the 6th day, dying on the 12th day. The daily counts of this rat were as follows:—

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	—	—	4	864

Day	8	9	10	11	12
Parasites per c.mm.	2196	4308	30,600	10,000	75,200

Secondly, a further experiment was tried, since it was considered that the parasites in one drop of spleen-pulp solution might be too numerous to count accurately, and that a few flagellate

trypanosomes might be contained therein, for although a most minute search was made for flagellates in the fluid before inoculation, yet a very few flagellates might have remained undetected. Accordingly, in the second experiment, 1 cubic millimetre of spleen-pulp solution in sodium citrate was inoculated intraperitoneally. Careful examination of samples of the solution used, both fresh and stained, showed no flagellate trypanosomes, but many rounded bodies. The actual fluid used for inoculation also showed no flagellate trypanosomes when microscopically examined fresh. The rat thus inoculated developed trypanosomiasis, parasites being found on the 8th day, and it died on the 18th day. The daily counts of this rat are appended:—

Day	1	2	3	4	5	6	7	8	9	10	11
Parasites per c.mm.	—	—	—	—	—	—	—	4	960	2800	12,000

Day	12	13	14	15	16	17	18
Parasites per c.mm.	49,200	18,200	6400	2120	20,600	50,240	128,000

The two rats inoculated with non-flagellate, latent bodies of *T. rhodesiense* showed incubation periods rather longer (5 to 7 days) than usual (2 to 4 days) with rats inoculated with flagellate *T. rhodesiense*.

The occurrence of non-flagellate bodies in the life-cycle of the parasites of sleeping sickness also explains recurrence of trypanosomiasis after it has apparently died out in an infected animal. In such cases the latent bodies are present in the host all the while in such organs as the spleen and bone marrow.

During these researches it has been found that flagellate trypanosomes (500,000 to 2,000,000 in number) inoculated into a rat or a guinea-pig can be detected in the peripheral blood-stream (in 1 cubic millimetre of blood) of the host for some ten to twelve hours, or even eighteen hours after inoculation. During and after this period, that is, during the incubation period of the parasite,

a few rounded forms of trypanosomes can be found in a cubic millimetre of peripheral blood.

Biot (1910) writes of the 'revivifying action' of physiological salt solution on trypanosomes (*T. lewisi*), especially in fluid from the liver of a rat dead six and a half days (kept in the cold, unopened). Biot does not explain the phenomenon. However, it is capable of explanation, for in the liver latent bodies of trypanosomes are present, which, under the relatively favourable environment of isotonic salt solution, flagellate and become typical trypanosomes.

In the treatment of trypanosomiasis by drugs, careful note must be taken of the occurrence of rounded, non-flagellate or latent forms of the parasite. A drug needs to be found which will either prevent the formation of rounded (latent) stages or disintegrate those latent bodies already formed. In this connection the work of B. Moore, Nierenstein, and Todd* on the combined use of salts of mercury and arsenic should be considered.

NOTE ON THE DEGENERATION OF TRYPANOSOMES

All flagellate trypanosomes do not become rounded and form latent, non-flagellate bodies, but some of them degenerate and die. The degeneration may take various forms—some become (*a*) somewhat irregular and almost amoeboid with pale-staining cytoplasm and vacuoles (figs. 44, 45), others (*β*) exhibit chromatolysis, wherein the nucleus becomes poor in chromatin and chromatoid granules occur in the cytoplasm (figs. 46-49), while others (*γ*) exhibit marked vacuolation (fig. 50). Such degenerating forms may be seen in various internal organs of the host, such as the lungs and spleen, especially during the period of formation of latent bodies.

It is also very probable that some of the latent bodies themselves die and do not flagellate, for some shrunken latent bodies, with undifferentiated contents, can be seen in the spleen.

* Ann. Trop. Med. and Parasitol. (1927), Vol. I, pp. 275-284.

SUMMARY AND CONCLUSIONS

1. Non-flagellate stages of trypanosomes, such as *T. gambiense* (Dutton) and *T. rhodesiense* (Stephens and Fantham), occur.
2. These non-flagellate stages ('latent bodies' of Moore and Breinl) are especially found in the lungs, spleen and bone marrow, during periods of decrease of trypanosomes in the peripheral blood of the host.
3. They are in process of formation at or near the time when the trypanosomes are most numerous in the peripheral blood. The formation of latent bodies takes place especially in the lungs, and they collect in the spleen and bone marrow of the host.
4. In the formation of non-flagellate stages, some of the cytoplasm and the flagellum of the trypanosome are disintegrated. The non-flagellate body contains the nucleus and blepharoplast (kinetocore) of the trypanosome.
5. Non-flagellate (latent) bodies can be seen to grow and flagellate, turning into trypanosomes, when placed in fresh, warm, uninfected blood.
6. Latent bodies of *T. rhodesiense*, inoculated into a rat, flagellate and produce trypanosomiasis.
7. The non-flagellate (latent) bodies of trypanosomes (*T. gambiense* and *T. rhodesiense*) are the post-flagellate stages of one generation of trypanosomes and the pre-flagellate stages of the succeeding generation of trypanosomes.
8. There is a life-cycle of trypanosomes (*T. gambiense* and *T. rhodesiense*) in Vertebrate hosts, comparable with those of *Cnithidia* and *Herpetomonas* in the alimentary tracts of various Invertebrates. The latent (relatively resistant) stages of trypanosomes occurring in Vertebrates are separate from, and in addition to, stages of the parasite which may occur in the Invertebrate carrier (for example, *Glossina*).
9. In the treatment of trypanosomiasis by drugs, careful note must be taken of the occurrence of rounded non-flagellate or latent forms of the parasite. A drug needs to be found which will either prevent the formation of rounded (latent) stages, or disintegrate those latent bodies already formed.

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EXPLANATION OF PLATE XXVII.

All figures were outlined with Abbé-Zeiss camera lucida, using 2 mm. apochromatic homogeneous immersion objective and compensating oculars 8 and 12 of Zeiss. Magnification 2,000 diameters approximately, except where otherwise stated.

The chief stains used were iron haematoxylin, Giemsa, or Romanowsky after wet fixation.

The figures represent *T. rhodesiense*, as seen in rats, except where otherwise indicated.

Fig. 1 represents the formation of latent (non-flagellate) body from trypanosome in heart blood.

Figs. 2-5 represents latent bodies, with remains of trypanosomes around or near, from heart (fig. 4) and lungs.

Figs. 6, 7.—Oval latent bodies, each with nucleus and blepharoplast (kinetocore), from lung of rat and peripheral blood of guinea-pig respectively.

Figs. 8-16.—Various forms of latent bodies—from liver, heart, spleen, lung (figs. 8-11); of *T. gambiense* in peripheral blood of rat (fig. 12); from peripheral blood (fig. 13), lung, and heart respectively.

Figs. 17-20.—Rounding, herpetomonad-like forms, all from peripheral blood of guinea-pigs. Fig. 19 of *T. gambiense*.

Fig. 21.—Broad trypaniform parasite from blood of guinea-pig.

Fig. 22.—Latent form of *T. gambiense* from lung of rat.

Figs. 23-24.—Possible division forms of latent bodies of *T. rhodesiense* from heart and lungs of rats. Fig. 24 magnified 1,350 diameters. These forms are rare.

Figs. 25, 26.—Pyriform latent bodies, each with nucleus and blepharoplast. Fig. 25, of *T. rhodesiense* from blood of guinea-pig; fig. 26, of *T. gambiense* from blood of rat.



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LATENT & OTHER FORMS OF
 TRYPANOSOMA GAMBIENSE & T. RHODESIENSE.

Figs. 27, 28.—Pyriform bodies of *T. rhodesiense* and *T. gambiense* (fig. 28) from peripheral blood of guinea-pig. In fig. 28 the flagellum is shown beginning to develop.

Figs. 29, 30.—Two pyriform non-flagellate forms from spleen of rat.

Fig. 31.—Round form of *T. gambiense*, with nucleus, blepharoplast and short flagellum along the edge of the body; from peripheral blood of rat.

Figs. 32-40.—Crithidia-like forms. Fig. 32, of *T. gambiense* from blood of rat; figs. 33-35, of *T. rhodesiense* from lung (fig. 33) and peripheral blood of rat; figs. 36-38, of *T. gambiense* from blood of guinea-pigs; figs. 39-40, of *T. rhodesiense* from blood of guinea-pigs. In fig. 37 note the basal granule ('blepharoplast' of Minchin) at the root of the flagellum, forming the centrosome of the kintonucleus.

Fig. 41 represents an almost trypaniform stage of *T. gambiense* from blood of guinea-pig. Note the peculiar position of the blepharoplast (kintonucleus).

Fig. 42 represents a stout trypaniform parasite from heart blood of rat.

Fig. 43 shows peculiar, large rounding form (from blood of a rat), exhibiting signs of division of its nucleus and blepharoplast.

Figs. 44, 45.—Slightly irregular parasites, very pale staining and vacuolated. Degenerating, as seen in detachment of flagellum, etc.

Figs. 46-49.—Degenerating trypanosomes, exhibiting chromatolysis, all from lungs of rats. Fig. 46 of *T. gambiense*, magnified 1,350.

Fig. 50.—Degenerating *Trypanosoma gambiense*, showing vacuolation, pale-staining cytoplasm and beginning of disintegration.